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Response of a Phagocyte Cell System to Products of Macrophage Breakdown as a Probable Mechanism of Alveolar Phagocytosis Adaptation to Deposition of Particles of Different Cytotoxicity

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The adaptation of the alveolar phagocytosis response to the quantitative and qualitative features of dust deposited during inhalation consists not only in enhanced recruitment of alveolar macrophages (AM), but also in adding a more or less pronounced neutrophil leukocyte (NL) recruitment as an auxiliary participant of particle clearance. The NL contribution to clearance is especially typical for response to cytotoxic particles (quartz, in particular). An important feature of the adaptation considered is the limitation of the number of AM and NL recruited when an efficient clearance can be achieved by a lesser number of cells due to increased AM resistance to the damaging action of phagocytized particles. The main mechanism providing the adequacy of the alveolar phagocytosis response is its self-regulation through the products of macrophage breakdown (PMB). In a series of experiments with intraperitoneal and intratracheal injections of syngenetic PMB into rats and mice, it was shown that these products stimulate respiration and migration of phagocytic cells, their dose-dependent attraction to the site of PMB formation with the predominant NL contribution, increasing with the increase of amount of PMB, the AM and NL precursor cells recruitment from reserve pools, and the replenishment of these reserves in the process of hemopoiesis. At least some of the above effects are connected with the action of the lipid components of PMB. The action of specialized regulative systems of the organism can modify the response to PMB, judging by the results obtained by hydrocortisone injection. Autocontrol of alveolar phagocytosis requires great care in attempts at artificial stimulation of this process, as an excessive cell recruitment may promote the retention of particles in lungs.

According to estimates based on comparison of calculated deposition of dust in miners' lungs with the actual amount found postmortem, about 98-99% of primarily deposited dust mass is cleared from the respiratory system (*1*). This impressive calculation allows us not only to explain why life is compatible with breathing in dusty air, but also leads us to two conclusions important for industrial hygiene. The first is that even small differences in the individual efficiency of the physiological mechanisms of self-clearance of lungs lead to very great differences in their dust retention. This is confirmed by the results of experiments on volunteers (*2*).

The second conclusion is that different factors which even slightly increase this efficiency of clearance could be of great practical importance. Although this conclusion seems self-evident, it requires certain reservations. We showed that at least one of the main mechanisms of particle clearance is a

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self-regulating process. There are grounds for treating attempts at artificial stimulation of this process with a certain caution.

Alveolar Phagocytosis as the Pulmonary Clearance Mechanism

Two combined physiological mechanisms play an important role in clearing the respiratory tract of practically insoluble particles: mucus transport, which is activated by ciliary activity, and phagocytosis of particles by cells on the free surface of the respiratory tract, mainly in the alveolar region.

Although the role of phagocytosis is confirmed only by circumstantial evidence, there remains little room for doubt. There is a direct relationship between the average number of free cells washed out of the respiratory tract of an animal and the individual efficiency of pulmonary clearance (*3, 4*). The lung tissue and, particularly, the regional lymph nodes retain more dust when the cytotoxic action of the particles on the phagocytizing cell is greater (*5-8*). The influence of poly (2-vinylpyridine *N*-oxide) (PVPNO), which increases the resistance of pulmonary macrophage to such action of quartz particles, at the same time decreases their retention in lungs and lymph nodes (*9-11*). This interconnection is proved by the fact that PVPNO does not significantly influence the retention in lungs of noncytotoxic particles (*12*). When the resistance of pulmonary macrophages to the damaging activity of quartz occurs on the background of nonspecific adaptation of the organism depending on physical training or repeated action of small concentrations of sulfur dioxide, the retention of quartz in lungs also decreases (*13*). On the contrary, enhanced degeneration of alveolar macrophages in rats exposed to long-term inhalation of the same quartz dust on the background of a per oral administration of excess fat was accompanied by increased quartz retention (*14, 15*).

Thus, a certain number of free phagocytizing cells and their functional stability seem to prevent penetration of dust particles into the lung tissue and lymphatics. It is supposed that only the nonphagocytized particles can penetrate, while the alveolar macrophage, already loaded with dust particles, is not able to migrate back through the alveolar wall (*16-21*).

We shall not dwell on the complicated and moot question of the nature of forces which cause this cell to move to the exit of the lung acinus into the zone of action of the "mucociliary escalator." This problem was discussed recently (*20, 21*). We think that the most probable way is the passive transfer of the macrophage with the liquid currents over the free surface of the lung acinus, no matter what the cause of the currents themselves is. The same force, apparently, provides for the removal of free particle from the acinus if the particle has not as yet penetrated into the lung interstitium. On the other hand, there is evidence to show that a particle which has already penetrated can rather quickly be ejected by short routes of lymphatic drainage into the free surface of a bronchiole already in the zone of mucociliary transport (*22, 23*). However, only some small proportion of particles which had penetrated remains in the lung tissue or moves with the lymph current into the intralung and regional nodes of the lymphoid tissue. Which of these fates awaits a particle deposited in the lungs seems not to be strictly determined, and phagocytosis of this particle on the free surface of the acinus appears to be a phenomenon which decreases the probability of penetration and retention of the particle in the lungs and increases the probability of its quick elimination. If this is really so, the ability of an organism to respond to the deposition in the alveoli of particles by the more active recruitment of phagocytic cells into the alveoli would be biologically expedient. Experimental data show that this is true, at least for mineral particles.

Adaptivity of the Alveolar Phagocytosis Response

Increasing the number of particles injected into the lungs increases the recruitment of alevolar macrophages and, while the lungs are eliminating the injected dose, the number of macrophages that can be washed out of the respiratory tracts is decreased (*3, 4*). Very useful improvements of this technique of quantitation of alveolar macrophages were introduced (*21, 24, 25*) and have confirmed the fundamental idea that mobilization of alveolar macrophages depends on the number of dust particles deposited in lungs. It was shown also that the response depends on the nature of deposited particles (*25*).

The study of the response of alveolar phagocytosis in rats under conditions of long-term inhalation of quartz dust was begun by Katsnelson et al. (*10, 26-29*). These studies, as well as our subsequent experiments, made use of an original technique (*3, 4*) based on a single lung washing. This was, however, supplemented by a cytological study of the residue of the centrifuged washings (*30*). The lungs were removed for washing, as a rule, 24 hr after the final exposure to dust or intratracheal injection which corresponds to the period of maximum recruitment of alveolar macrophages (*25*).

We discovered and repeatedly reproduced the

following peculiarities of the response of alveolar phagocytosis to the deposition of quartz particles in lungs.

First, the numbers of recruited alveolar macrophages (AM) are considerably higher with quartz than with the deposition of dust particles of low cytotoxicity (e.g., ferrous oxide or titanium dioxide) which agrees with the results obtained elsewhere (*8*).

Second, highly significant differences are found in the number of neutrophile leukocytes (NL) in the same washings.

The increased ratio of the number of NL to the number of AM is typical of the response of lungs to inhalation as well as to the intratracheal injection of quartz.

Third, both above-mentioned peculiarities, especially the increase of the mean NL/AM ratio, were more pronounced the sharper the cytotoxic action of quartz on the alveolar macrophages was. For example, both AM and NL counts in washings and the NL/AM ratio were considerably lowered after subcutaneous injections of PVPNO which reduced the average percentage of degenerated AM (in comparison to rats exposed to dust in the same chamber, but not injected) by a factor of more than three (*10*). The same experiment showed that retention of quartz in lungs during 6 months exposure for rats given PVPNO was lowered by a factor of 3.25. This illustrates another feature of adaptivity of the alveolar phagocytosis response which is seldom noticed but is of considerable importance: when stability of functional and morphological integrity of the macrophage allows the organism to perform efficient phagocytosis of particles by a lesser number of cells, their recruitment is automatically curtailed.

The important role of the number of degenerated macrophages is seen when analyzing not only the inter-group, but also the inter-individual differences in alveolar phagocytosis. Thus, we showed (*31*) that after long-term inhalation of quartz dust there is a dependence between the percentage of degenerated AM in every rat (x) and the NL/AM ratio for the same rat (y) which can be described by the equation

$$y = 0.2 + 0.035\,x - 0.001x^2 + 0.00001x^3$$

A similar dependence

$$y = 0.31 - 0.04x + 0.02x^2 - 0.00002x^3$$

appeared in control rats. This suggests the idea that the cause of AM breakdown is less important for the development of the alveolar phagocytosis response than the very fact of this breakdown.

Not only the differences of the response of the organism to the same dust, but also the peculiarities of the reaction to different dusts evidently depend on the average percent of the degenerated AM. For example, after the intratracheal injection into rats of 5 mg TiO_2 in 1 ml of normal saline the percentage of degenerated macrophages (18.1 ± 2.4, mean ± SE) was only slightly increased in comparison with the respective value for rats which received 1 ml of normal saline (12.2 ± 0.8%, $p < 0.05$) while an analogous injection of 5 mg of quartz gave 54.2 ± 5.3% of clearly degenerated AM ($p < 0.001$). The mean NL/AM ratio was, respectively, 0.28 ± 0.07, 0.24 ± 0.03, and 1.71 ± 0.15 ($p < 0.001$). Still earlier it was shown that an analogous difference exists for the reaction to quartz, and Fe_2O_3, or the same quartz treated by trimethyltrichlorosilane which sharply decreases its cytotoxicity (*29*).

Little attention has been paid to the contribution of NL to the reaction of alveolar phagocytosis when nonmicrobial particles are deposited in the lungs (*32-36*). The attention of researchers studying the phagocytic response to different mineral particles is, as a rule, concentrated on macrophages. Migration of NL into the respiratory tract, if noted at all, is considered only as a symptom of "inflammation" (*37*). However, we must note that experiments with quartz dust performed not only by us but also by other researchers showed an unusually low percentage of AM in the free cell population of the respiratory tract with the increase in the NL percentage (*38-40*).

Macrophage Breakdown and Control of Phagocytosis Response

Results discussed above gave reason to assume that the macrophage breakdown is a signal to recruit an increased number of alveolar macrophages and a still greater number of neutrophile leukocytes into the respiratory tract, the mediator of this signal being some substances formed or released during AM breakdown (*28, 29*). Similar hypotheses, based on other, usually speculative assumptions, were put forward by some other researchers as well (*41, 42*).

We performed a number of experimental studies (*27, 43-46*), the results of which allow us to consider such a hypothesis substantiated enough. Experiments were performed on Wistar rats and CBA, C57BL, and BALB/c mice. The products of syngenetic macrophage break-down (PMB) were injected intratracheally or intraperitoneally. The PMB used for this were obtained aseptically by triple freezing and thawing the peritoneal exudate cells obtained 45 hr after intraperitoneal injection of a sterile mineral oil or normal saline.

Influence of PMB on Phagocytising Blood Cells

Although there remains some vagueness in the question of tissue macrophage origin in general and pulmonary macrophage origin in particular there is little doubt today that all these cells in the final count have a bone-marrow origin. This question has already been discussed in this journal and other recent reviews (*21, 48, 49*). We can refer also to conclusive results of observations of chromosome changes in macrophages of bronchopulmonary lavages in patients after a successful bone marrow transplantation from persons of opposite sex (*50*). Along with this, convincing experimental data show that the local cell reserve — the interstitial precursor cell pool — may be an immediate source of AM recruitment to the alveoli (*47, 51-54*). This reserve provides, on the one hand, a certain independence of enhanced AM mobilization as a response to a short-term challenge from the systemic increase of hemopoiesis, which, it seems, is also characteristic for macrophages of other organs (*55*), and, on the other hand, creates necessary conditions for maturing a functionally valid AM alongside with its gradually acquiring a number of morphological and biochemical features.

These considerations give prime importance to the question of the influence of PMB on the production of granulocytes and monocytes by the hematopoietic tissue and on their mobilization by blood. The results of the experiment, in which rats received by intraperitoneal injection three times in 3 days a dose of PMB corresponding to 1.5×10^8 of destroyed macrophages per 100 g. body weight, are shown in Table 1. The changes in the cell composition of bone marrow show increased maturing of neutrophils and monocytes. Although we could not discover any enhancement of endogenous respiration of bone marrow, the influence of PMB considerably lowered the critical P_{O_2} level which limits the cell breathing. That shows the ability of cells to use oxygen more efficiently. (*56*). Shifts similar in many respects were caused by triple injection of quartz suspension intraperitoneally by 30 mg and, to a lesser degree, by similar injection of TiO_2. This can be explained by formation of PMB *in vivo* in quantities depending on the cytotoxicity of dust.

Figure 1 shows the change of the average NL and monocyte counts in the peripheral blood of rats after a single injection of the same dose of PMB. The original sharp increase of the NL count is probably connected with neutrophiles mobilization from different reserve pools, but the subsequent steady leukocytosis, both neutrophilic and monocytic, can be connected with the increase of granulocyte and monocyte formation. To analyze the action of the same dose of PMB on the hemopoietic tissue we used also the experimental technique (*57, 58*), which allows us to estimate the content of multipotent stem cells from the number of colony-forming units in the spleen (CFU-S). Typical results of these experiments are shown in Table 2. Analogous results were obtained on mice of other strains.

Although PMB does not exercise any direct stimulating influence on the proliferation of stem cells *in vitro*, its injection into mice bone-marrow donors showed the increase of the number of CFU-S with a shift of differentiation to granulocytic colonies. It is interesting to note that a similar shift was not observed when erythrocyte breakdown products were injected, although the general number of colonies was increased in this case as well (*59*).

Influence of PMB on Recruitment of Phagocytising Cells

The cytotoxic influence of mineral particles causes an influx of granulocytes into the respiratory tract as demonstrated in an experiment with triple IP injection of 30 mg of quartz or TiO_2. The exudate obtained 24 hr after the third injection of the titanum

Table 1. Some cytological and polarographic characteristics of bone marrow cells of rats after three intraperitoneal injections of PMB, quartz, or TiO_2.

	After intraperitoneal injection of			
	Normal saline	PMB	Quartz	TiO_2
O_2 consumption, nAO_2/min per 10^6 cells	3.2 ± 0.3	3.14 ± 0.4	3.72 ± 0.8	2.95 ± 0.2
Critical level P_{O_2} mm Hg	38.3 ± 3.8	27.8 ± 3.2[a]	28.4 ± 2.1[a]	29.4 ± 4.5
Mature neutrophil count, %[b]	62.0 ± 4.3	77.0 ± 5.0[a]	82.5 ± 4.7[a]	67.0 ± 2.5
Monocyte count, %[c]	0.8 ± 0.1	2.1 ± 0.4[a]	1.3 ± 0.1[a]	1.3 ± 0.2[a]

[a]Values statistically significantly different ($p < 0.05$) from the corresponding ones after injection of normal saline.
[b]Percent of mature forms in all the neutrophil series cells.
[c]Percent of monocytes in all the bone marrow cells (counting 1000 cells in each smear).

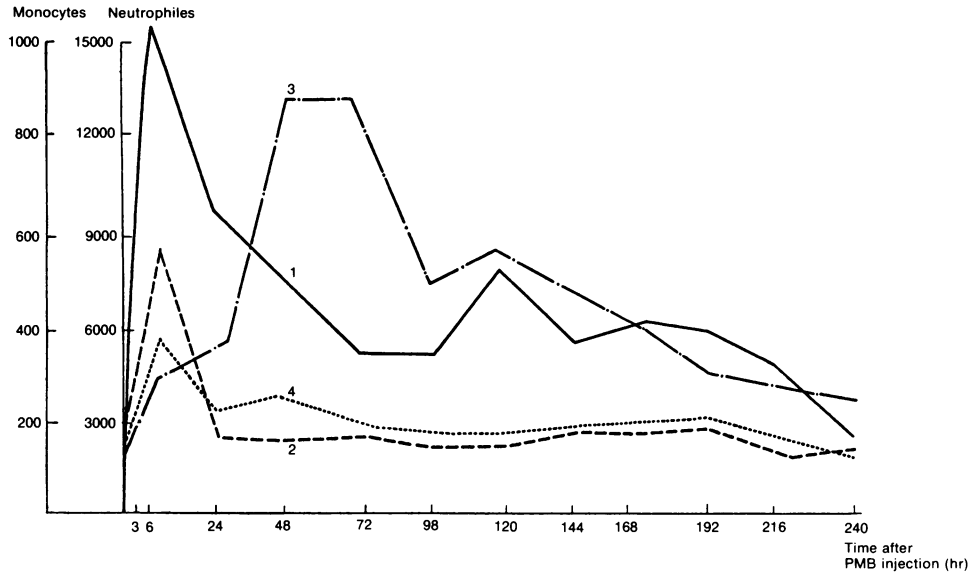

FIGURE 1. Effect of PMB on neutrophile and monocyte counts in rat peripheral blood: (1) neutrophiles after injection of PMB; (2) neutrophiles after injection of normal saline (control); (3) monocytes after injection of PMB; (4) monocytes after injection of normal saline (control). Differences between experimental and control series statistically significant ($p < 0.05$) for neutrophiles at all times of testing except the last; for monocytes from 24 to 144 hr.

dioxide suspension contained only 15.5 ± 1.9% of granulocytes (relative to all cells). That did not differ from the exudate composition after triple injection of the normal saline. At the same time, after a similar injection of quartz suspension, this figure rose to 32.0 ± 2.8% ($p < 0.001$). Clearly degenerated cells constituted, correspondingly, 7.0 ± 1.7% and 50.0 ± 2.4% of all the exudate macrophages ($p < 0.001$). Such attraction of granulocytes to the site of macrophage breakdown was modelled in intraperitoneal injection of artificial PMB in the dose mentioned in the above section. In this case, the granuolocytes rose to 52.3 ± 3.3% ($p < 0.001$). However, neither the rats exposed to the titanium dioxide dust by inhalation nor those who were not exposed to it showed any change in the total number of cells in the lung washings or in the NL/AM ratio under the influence of intraperitoneally injected titanum dioxide or quartz suspension or the artificial PMB.

Thus, along with the systemic action of PMB on

Table 2. Effect of PMB on the CFU-S in the bone marrow and spleen of CBA mice.

Index	Tissue and Treatment	Injection of normal saline	Injection of PMB	Significance[a]
Number of CFU-S	Bone marrow of the femur of donor mice, PMB or normal saline injected	895.4 ± 281.2	2827.4 ± 616.4	$p < 0.01$
E/G ratio[b]	"	4.6 ± 1.4	1.4 ± 0.3	$p < 0.05$
Number of CFU-S	Spleen of donor mice, PMB or normal saline injected	2520.0 ± 362.9	7585.5 ± 1221.0	$p < 0.001$
E/G ratio	"	3.4 ± 1.1	1.8 ± 0.3	NS
Number of colonies	Spleen of recepient mice previously treated with PMB or normal saline after injection of 10^5 cells of bone-marrow of intact donor mice	7.3 ± 1.0	29.9 ± 0.6	$p < 0.001$
E/G ratio	"	2.9 ± 0.5	0.6 ± 0.05	$p < 0.001$
Number of colonies	Spleen of recepient mice after injection of 10^5 cells of bone marrow of intact donors preincubated with PMB	7.0 ± 1.0	8.3 ± 1.2	NS

[a]NS denotes deviation from control statistically not significant ($p > 0.05$ according to Student's t-test).
[b]E = erythroid colonies; G = granulocyte colonies.

producing phagocytizing cells, one can observe a local effect on these cells. It is still more pronounced when PMB are injected intratracheally. Many such experiments were performed with different PMB doses. Control rats in this case were injected intratracheally with 1 ml of normal saline. The analysis of lung washings was conducted after 24 hr. In two experiments the animals were exposed also to the TiO_2 dust which they inhaled in a chamber at 50 mg/m^3 or 85 mg/m^3 concentration 5-6 hr per day for four consecutive days, and the PMB was injected in 3×10^8 doses immediately after the fourth inhalation, or in half doses, after the second and fourth inhalations. (Here and further on we give the PMB doses per rat in units of about 200 g, corresponding to the number of cells destroyed; the PMB concentrations are given in the same units per milliliter of medium.) All these experiments without exception showed increased recruitment of phagocytizing cells into the respiratory tract under the influence of PMB, sufficiently large doses causing attraction of both AM and NL, while smaller ones caused only NL attraction. However, even when the doses were larger, the number of NL showed a greater increase in comparison with the control than the number of AM, which resulted in a higher NL/AM ratio at all doses than in the control groups. This ratio increased with an increase in dose.

Such dose-effect relationships were analyzed in a special series of experiments while simultaneously testing the following doses of PMB: 0, 1×10^6, 2×10^6, 5×10^6, 1×10^7, 2.5×10^7, 5×10^7, 1×10^8, 1.5×10^8, or 3×10^8. A statistically significant increase of number of NL in the respiratory tract in comparison with the "zero" dose was obtained already with the 1×10^6 dose. With the increase of the PMB dose the number of NL increased according to the equation,

$$y = 0.24 + 0.02x - 0.00005x^2$$

However, even the 5×10^6 dose did not cause an increase in number of AM in the same washing in comparison with the control level ($0.82 \pm 0.06 \times 10^6$). Some increase in the number of AM ($1.55 \pm 0.52 \cdot 10^6$) was first noticed with a dose of 2.5×10^7, but the increase proved to be statistically significant only at doses of 5×10^7 or higher. At the same time, this index practically coincided (from $1.78 \pm 0.49 \times 10^6$ to $1.86 \pm 0.36 \times 10^6$) at doses from 5×10^7 to 1.5×10^8, i.e., when the PMB amount in lungs was increased threefold. A further double increase (3×10^8) showed another doubling of the number of AM in washings (up to 3.70 ± 0.92).

Thus, unlike the NL attraction from blood into the respiratory tract, which increases almost linearly with the increase of attractant amount, the AM recruitment is governed by the action of PMB rather than by a trigger mechanism. One cannot eliminate the possibility of the existence of different sources and/or mechanisms of additional mobilization of AM into the alveoli, each of which has its own PMB dose range or its particular threshold dose. For example, we can suppose that the background number of free AM is provided mainly by migration of mature macrophages from the interstitial pool (*21, 52, 53*). It is hard to say to what degree this background migration is connected with the attraction of cells by breakdown products of the macrophages with evident degeneration which are always present in the respiratory tract. The above-mentioned dependence of the number of AM in control rats on the percentage of such macrophages indirectly proves that they also exhibit such an attraction, depending on the PMB dose. In this case an additional PMB injection in small doses may simply fail to give a noticeable effect on the background of individual fluctuations of the amount of endogenous PMB. However, the fact that high enough doses not only make this effect noticeable, but also act in a certain range on the "all-or-nothing" principle, allow us to think of "ejection" from the same pool of some subpopulation of predecessor cells which have not yet reached the mature stage. The next threshold dose either acts in a similar way on a still less mature subpopulation or else causes a direct attraction of mononuclear phagocytes from the blood into the alveoli. The supposition about the possible existence of different sources of AM recruitment in the state of relative rest and as a response to a pronounced challenge on the part of deposed particles was expressed elsewhere (*21*).

It was shown that both the quantity of and the ratio between different phagocytes in the lung washings are reminiscent of the picture typical for the quartz dust reaction the more, the greater the PMB dose is. Thus, for a dose range from 0 to 2×10^6, the average number of AM was $0.82 \pm 0.06 \times 10^6$; the average number of NL was $0.12 \pm 0.006 \times 10^6$, and the average NL/AM ratio was 0.15 ± 0.018; for a $5 \times 10^6 - 5 \times 10^7$ dose range, the respective values were $1.33 + 0.018 \times 10^6$, $0.83 \pm 0.138 \times 10^6$, and 0.54 ± 0.013; for a $1 \times 10^8 - 3 \times 10^8$ dose range, respective values were $2.19 \pm 0.338 \times 10^6$, $2.55 \pm 0.401 \times 10^6$, and 1.18 ± 0.069.

Table 3 shows the data of one of the experiments in which PMB was injected both to unexposed rats and to those who previously inhaled the TiO_2 dust. This dust, which is of little cytotoxicity and even sometimes called inert, caused, in combination with additional PMB, the alveolar phagocytosis reaction of the response to quartz dust type. It is also easy to see that even without an addition of exogenous PMB it caused (in comparison with the control) a

Table 3. Effect of a single intratracheal injection of PMB on the number of free cells in the respiratory tract of rats.

Exposure	Intratracheal injection	Number of cells in washing × 10^6			NL/AM
		All cells	AM	NL	
No exposure to dust	Normal saline	4.47 ± 0.51	3.02 ± 0.53	0.39 ± 0.14	0.13 ± 0.03
	PMB	12.89 ± 1.42	5.31 ± 0.51	5.09 ± 0.66	0.96 ± 0.01
		$p < 0.01$	$p < 0.01$	$p < 0.001$	$p < 0.001$
Four exposures to TiO_2, 5 hr (TiO_2 concn = 50 mg/m³)	Normal saline	8.66 ± 1.20	6.46 ± 0.71	1.56 ± 0.77	0.21 ± 0.10
	PMB	16.47 ± 1.08	9.24 ± 0.59	6.04 ± 1.09	0.75 ± 0.20
		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.001$

phagocytic reaction with a certain increase in the NL/AM ratio, although, as stated above, this was not observed with a single intratracheal injection of 5 mg of a TiO_2 suspension. The inhalation experiment gave the mean value of clearly degenerated AM in control rats of 9.6 ± 0.7% while in animals exposed to the TiO_2 dust it was 16.0 ± 1.2% ($p < 0.001$).

So, whether we speak of the cytotoxic action of quartz, of a considerably less pronounced and—probably having other causes — damaging action on a cell of other dust particles, of a breakdown of part of the AM caused by different "natural" reasons in the respiratory tract of control rats, or of an intratracheal injection of breakdown products caused by a rough physical action on the macrophages, we observe in every case a change, similar in principle, of the free cell population of the respiratory tract, the degree of which evidently depends on the number of disintegrated macrophages.

Influence of PMB on the Functional Activity of Cells Able to Phagocytize

Attraction of macrophages and neutrophils to the site of formation or injection of PMB can be imagined as a result of motion of these cells against a concentration gradient of the factor which stimulates their migration. In fact, we found by means of a technique used for quantitative evaluation of macrophage migration (*60*) that PMB increases it. This effect depends on the PMB concentration in the medium. The experiments were performed with peritoneal macrophages of C57BL mice and the breakdown products of the same cells, the PMB being previously centrifuged for 20 min at 6000 rpm. Under the influence of the supernatant in concentrations corresponding to 5×10^6 and 1×10^7 PMB, the increase in migration area in comparison with the control was equally small and statistically insignificant (respectively, 1.15 ± 0.12 and 1.12 ± 0.11 times), but at the 5×10^7 concentration it was significant ($p < 0.05$) and reached 1.67 ± 0.37 times. The influence of PMB on the NL migration was studied by means of another model which had a cultured film of human leukocytes as a test object (*61, 62*). Under the influence of the supernatant of the rat PMB in a 1×10^6 concentration the increase of migration area in comparison with the control was small and statistically significant (1.15 ± 0.24 times), but with increased concentration the effect rose up to 7.2 ± 2.3 ($p < 0.05$) at $1.5 \cdot 10^8$. With the same dose of whole PMB, the migration increase was so sharp that it could not be quantitatively estimated by the present technique.

The consumption of oxygen by the alveolar and peritoneal macrophages (PM) was studied polarographically. The endogenous respiration of both AM and PM was not limited by a lack of substrate because the addition of a $10^{-2}M$ succinate did not increase the oxygen consumption. With introduction of whole PMB to the cell-free medium, the oxygen was not consumed. Thus, we have grounds to consider the observed increase in oxygen consumption under the stimulating action of PMB in 1×10^7 concentration on the average of 31.8% for PM (from 2.22 ± 0.07 to 2.92 ± 0.17 nA O_2/min per 10^6 cells, $p < 0.001$) and of 21.6% for AM (from 3.66 ± 0.2 to 4.42 ± 0.24, $p < 0.02$) to be a result of a real enhancement of macrophage metabolism. When the number of cells in a polarograph unit was equal, the PMB stimulating effect was equal for AM and PM, in spite of the known peculiarities of aerobic metabolism of AM (*52, 63-66*).

The decrease of the number of dust particles phagocytized by the single AM in lung washings from rats which received PMB (Table 4) seems to be paradoxical in comparison with all the above evidence of functional stimulation of the macrophage under the direct influence of PMB. This effect can be seen by the average number of phagocytized particles in one active AM in rats which had inhaled dust only from the unfiltered ambient atmosphere; by the decrease of percent of active AM containing too many particles to count in rats which had in two

Table 4. Effect of intratracheal injection of PMB on the activity of phagocytosis of dust particles *in vivo*.

Exposure	Intratracheal injection	Active AM, %	Average number of particles in "active" AM	Active AM with undefinable number of particles, %	Average number of particles in NL
None	Normal saline	70.3 ± 2.9	3.84 ± 0.99	Absent	1.67 ± 0.52
	PMB	49.1 ± 2.0	2.85 ± 0.54	Absent	1.50 ± 0.49
		< 0.001	$p < 0.05$		NS
TiO_2[a]	Normal saline	85.8 ± 1.9	Could not be counted	30.2 ± 5.7	1.43 ± 0.55
	PMB	77.0 ± 1.7	Could not be counted	14.9 ± 4.2	1.86 ± 0.55
		$p < 0.05$		$p < 0.05$	NS
TiO_2[a]	Normal saline	72.4 ± 0.7	Could not be counted	11.1 ± 1.2	1.57 ± 0.39
	PMB	60.7 ± 1.8	Could not be counted	0.6 ± 0.2	1.82 ± 0.41
		$p < 0.001$		$p < 0.001$	NS

[a]Two different experiments.

independent experiments inhaled the TiO_2 dust in a chamber at different concentrations; and by the lowering of the percent of active (i.e., containing at least one visible dust particle) cells of the general AM number in both groups.

One can suggest the following explanation to such a discrepancy between the stimulating action of PMB and the observed lowering of phagocytic activity of the free macrophage population of the respiratory tract. The intratracheal injection of PMB increases the recruitment of insufficiently mature cells from the interstitial pool of precursors and, possibly, from the pool of circulating monocytes, i.e., the contribution of these cells into the general population of macrophages entering the alveoli increases. This leads to the increase of the percentage of free AM which possess insufficient phagocytic activity in comparison with the mature AM functioning most efficiently in the peculiar conditions of free alveolar surface. The appearance of "young" and metabolically less active AM had been described also with quartz dust deposited in the lungs (*67*).

The intratracheal injection of PMB simulates one of the results of deposition of cytotoxic dust particles in lungs. In real conditions of inhalation of such particles and with PMB formation as a result of damage to phagocytizing AM by these particles the above-mentioned effect means a decrease of the mean number of these particles in a single AM of subsequent generations whose recruitment would be connected with the breakdown of the first AM "echelons." This means a reduction of the cytotoxic action of dust on AM and, consequently, an increase of the probability of AM retaining its integrity and fulfilling its role in pulmonary clearance. The seemingly unfavorable PMB effect must get another evaluation in the light of these ideas.

Further, due to a general increase in AM and NL counts, the total phagocytosis activity evaluated by the number of particles observed in a phagocytized state is not lowered but even considerably increased. This also contributes to the increase of clearance efficiency. In fact, after four daily dust exposures rats received on the second and fourth day 1 ml of normal saline intratracheally and were killed after 24 hr; the lungs of these rats showed on the average, 250.6 ± 44.0 μg Ti. Rats injected similarly with 1.5×10^8 PMB showed only 151.0 ± 21.4 μg ($p < 0.05$).

Thus, the favorable influence of PMB on cellular pulmonary clearance mechanism, which serves as a partial compensation for the unfavorable influence connected directly with the AM breakdown, consists in providing an effective engulfing of a greater total number of particles along with "defense" reduction of loading a single macrophage by these particles. Both are favored by a partial redistribution of the total dust load in the sharply increased NL count. Calculation based on data on rats not exposed in the dust chamber shows that under the influence of PMB the total sum of phagocytized particles increased 1.71 times ($p < 0.01$) while the contribution of neutrophil phagocytosis rose from 7.4% to 50.5%!

We have, therefore, every reason to believe that NL participation in alveolar phagocytosis is neither a chance phenomenon, nor some "inflammatory reaction" not connected with the physiological mechanisms of particle clearance, but is, in fact, one of such mechanisms naturally taking part in clearance, the necessity of such mechanism being the higher, the more cytotoxic are the particles deposited in lungs. This increase in demand is wholly answered by the increase of NL contribution into free cell population with an increase of the cytotoxic effect of real particles or the dose of PMB injected.

Table 5. Alveolar phagocytosis response 24 hr after the intratracheal injection of different products.[a]

Experiment number	Intratrachael injection	Number of cells washed out of lungs × 10^6			NL/AM ratio
		All	Macrophages (M)	Neutrophils (N)	
1	Normal saline	0.66 ± 0.07	0.59 ± 0.06	0.014 ± 0.003	0.024 ± 0.006
	PMB	2.20 ± 0.10*	0.88 ± 0.11*	1.21 ± 0.13*	1.36 ± 0.23*
	PMB residue	1.17 ± 0.11	0.45 ± 0.08	0.65 ± 0.08*	1.44 ± 0.31*
	PMB supernatant	1.28 ± 0.05*‡	0.68 ± 0.10	0.50 ± 0.08*‡	0.73 ± 0.16*
	PMB supernatant after incubation with SiO_2	1.10 ± 0.13†‡	0.70 ± 0.10	0.31 ± 0.04*†‡	0.44 ± 0.08*
	PMB supernatant under PVPNO protection	1.49 ± 0.09*†	0.84 ± 0.10*	0.50 ± 0.05*†	0.60 ± 0.09*
	Lipids extracted by Folch mixture plus Tween 20	15.20 ± 2.98*	2.74 ± 0.96*	11.48 ± 2.38*	4.19 ± 1.71*
	0.2% Tween 20 in normal saline	0.72 ± 0.09	0.55 ± 0.07	0.10 ± 0.02*	0.18 ± 0.04
2	Normal saline	0.95 ± 0.13	0.91 ± 0.13	0.013 ± 0.005	0.014 ± 0.006
	0.2% Tween 20 in normal saline	1.30 ± 0.09*	1.19 ± 0.09	0.03 ± 0.013	0.025 ± 0.011
	Serum 1:10	0.99 ± 0.18	0.90 ± 0.16	0.018 ± 0.006	0.020 ± 0.008
	PMB supernatant	2.59 ± 0.49*	1.92 ± 0.43*	0.53 ± 0.24*	0.28 ± 0.07*
	Supernatant of the destroyed muscle	1.00 ± 0.07	0.83 ± 0.08	0.11 ± 0.05	0.13 ± 0.06
	Lipids extracted by Folch mixture plus Tween 20	43.28 ± 9.27*	7.79 ± 2.80*	33.76 ± 7.63*	4.33 ± 1.84*
	Lipids extracted by Folch mixture plus serum	25.70 ± 10.40*	8.95 ± 3.67*	15.42 ± 7.20*	1.72 ± 1.07*
3	Normal saline	0.94 ± 0.13	0.88 ± 0.12	0.011 ± 0.002	0.013 ± 0.002
	PMB	3.38 ± 0.23*‡	1.68 ± 0.13*‡	1.49 ± 0.12*‡	0.89 ± 0.10*‡
	Residue of PMB after ether treatment	1.57 ± 0.17*‡	1.19 ± 0.13*‡	0.28 ± 0.04*‡	0.24 ± 0.04*‡
	Lipids extracted by ether, and Tween 20	37.03 ± 19.13*	15.90 ± 8.25*‡	18.82 ± 9.75*	1.18 ± 0.87*

[a]Pairs of values compared at $p < 0.05$ in the text are denoted by the same superscript (*, †, ‡); symbols the superscript symbol (*) denotes all values differing from the control at $p < 0.05$ (by Student's t-test).

[b]The PMB dose in experiments 1 and 2 corresponds to 1.5 × 10^8 destroyed cells, and in experiment 3 to 7.5 × 10^7 destroyed cells; the lipid dose always corresponds to the lesser of these PMB doses.

Role of the Lipid Fraction of PMB in the Control of Alveolar Phagocytosis

The supernatant obtained under the conditions described above by centrifuging PMB and the residue washed three times with normal saline showed, when injected intratracheally to rats, practically the same NL and AM attraction. Thus the recruiting activity of PMB is divided into two approximately equal parts by centrifuging, this half-activity being subthreshold for AM recruitment in some, but causing both effects in other experiments (Table 5). Note that the whole product proved more active than the supernatant in the estimation of the PMB action on migration *in vitro* as well. In both cases one may suppose phagocytizing of cell debris and additional lysis leads to freeing of the same acting agent which determines the activity of the supernatant.

The residue, as it was found, contains 1.5 times more protein than the supernatant, while the total lipids and different lipid fractions are divided equally between them. The supernatant obtained by analogous centrifuging of skeletal rat muscle which was first ground and then frozen and thawed three times did not influence the AM count when injected intratracheally, but gave a small increase of the NL count (Table 5). The injected dose of this product was equivalent in protein to the dose of the PMB supernatant which caused evident recruitment of both AM and NL. The total lipid content of this PMB dose was 17.7 times that in the muscle product. Let us note that the same product in a concentration which was equivalent in protein to a stimulating PMB concentration did not cause any change in oxygen consumption by peritoneal macrophages when introduced into the polarographic unit.

Even an evidently incomplete lipid extraction from PMB (by ether, on cooling and shaking) gives a residue causing a less pronounced recruitment of NL and AM than the undefatted PMB (Table 5). The lipids extracted by the same method or fully extracted from PMB (by Folch technique), and later

reemulsified with Tween 20, proved much more active in this respect than corresponding doses of PMB. Even a simple addition of serum proteins to these lipids (instead of Tween-20) somewhat reduces this effect. We cannot exclude the possibility that the paradoxically high activity of extracted macrophagal lipids is connected with a fuller freeing of lipids from protein complexes than that during simple macrophage breakdown. However, when we studied the macrophage migration *in vitro,* the same lipids proved to be only just as active stimulants of migration, as corresponding quantities of PMB supernatant. The stimulating action of triglycerides on macrophages *in vitro* was also described independently (*68*).

On the whole, all these data agree with the supposition (put forward elsewhere) that some lipid substances freed during macrophages breakdown under the action of silica play an important role in stimulation of alveolar phagocytosis (*41*).

Macrophagal Lipids and Quartz Particle Phagocytosis

Inhalation of quartz dust causes a considerable increase of sudanophilic inclusions in pulmonary macrophages, especially when the intake of fat into the stomach of rats is increased (*14, 16, 69*). This shift may be connected with the stimulation of the lipopectic function of the macrophage. We showed that intraperitoneal injection of quartz does not influence the number of sudanophilic inclusions and the content of different lipids in peritoneal macrophages, i.e., in cells which do not take part in the process of lipopexia. The influence of quartz on pulmonary macrophages can also be explained by the stimulating action of the PMB. We showed in a separate experiment that 24 hr after intratracheal injection of the PMB supernatant the percentage of AM containing an increased number of sudanophilic inclusions rises sharply.

It is natural to suppose that the breakdown of such AM under the influence of quartz particles must lead to formation of PMB with an increased lipid content. The stimulating action of the PMB on the mobilization of phagocytic cells is related to the PMB lipids.

Thus, a positive feedback between the cytotoxic action of quartz and the phagocytotic response of the lungs is realized not only through an increased amount of PMB, but also further through an increase in PMB activity (Fig. 2). One more positive feedback, shown in the same scheme, reflects the earlier proved (*15*) enhancement of alveolar macrophage degeneration caused by inhaled quartz in rats, which had, due to long-term excessive fat administration PO, an increased sudanophilic inclusion content in AM.

Together with this, there soon appears a negative feedback, the essence of which is not only in clearance, stimulated by the same PMB of the cytotoxic particles from the lung, but even earlier, in a redistribution of the particles between less mature AM and NL which promotes the weakening of the cytotoxic effect. As a result, the number of newly formed PMB begins to decrease gradually, which leads to a gradual lowering of the number of cells again recruited to replace those cleared from the respiratory tract.

Because of the intrinsic lag involved in this adaptation mechanism, the unfavorable influence of increased AM breakdown on the elimination of particles from the lungs cannot be fully compensated for. Together with this, a sharply enhanced passage of cells to the alveolar surface, drained by a considerably smaller surface of the acinus orifice, creates conditions for a stasis of a kind and thereby for detention of even the phagocyted particles (*71*). In fact, the hyperstimulation of the AM and NL recruitment by some biogenic preparations did not speed up, but rather slowed the elimination of ^{60}Co particles injected intratracheally, although a more moderate stimulation gave a favorable effect (*30*). Two long-term experiments showed that similar enhancement of quartz retention in lungs is caused also by a considerable enhancement of the alveolar phagocytosis response on the background of acclimatization of rats to moderate periodical coolings (*13*).

Autocontrol and Control

In these experiments the AM and NL recruitment was enhanced in spite of the fact that cold acclimatization increased the resistance of AM to the cytotoxic action of quartz, i.e., the mean percentage of degenerated AM was lowered, which fully corresponded to the lowering of the NL/AM ratio. Meanwhile, in other cases of induced increase of the AM resistance (when the rats were physically trained, exposed to long-term inhalation of sulfur dioxide, or injected with poly(vinylpyridine *N*-oxide) (PVPNO) or a pharmological adaptogen of the benzimidazole derivative series) in our laboratory we observed not only a lowering of this ratio, but also a general decrease of the number of recruited cells (*13, 27*) which can be easily explained by a decrease in the PMB formed. Acclimatization to cold in rats not inhaling quartz did not by itself cause any recruitment of cells into the respiratory tract. Consequently, there can exist some other mechanisms of control, besides the autoregulation of the alveolar phagocytosis through PMB and sometimes even seemingly against it.

A possible increase in macrophage recruitment may occur under the influence of atropine, epineph-

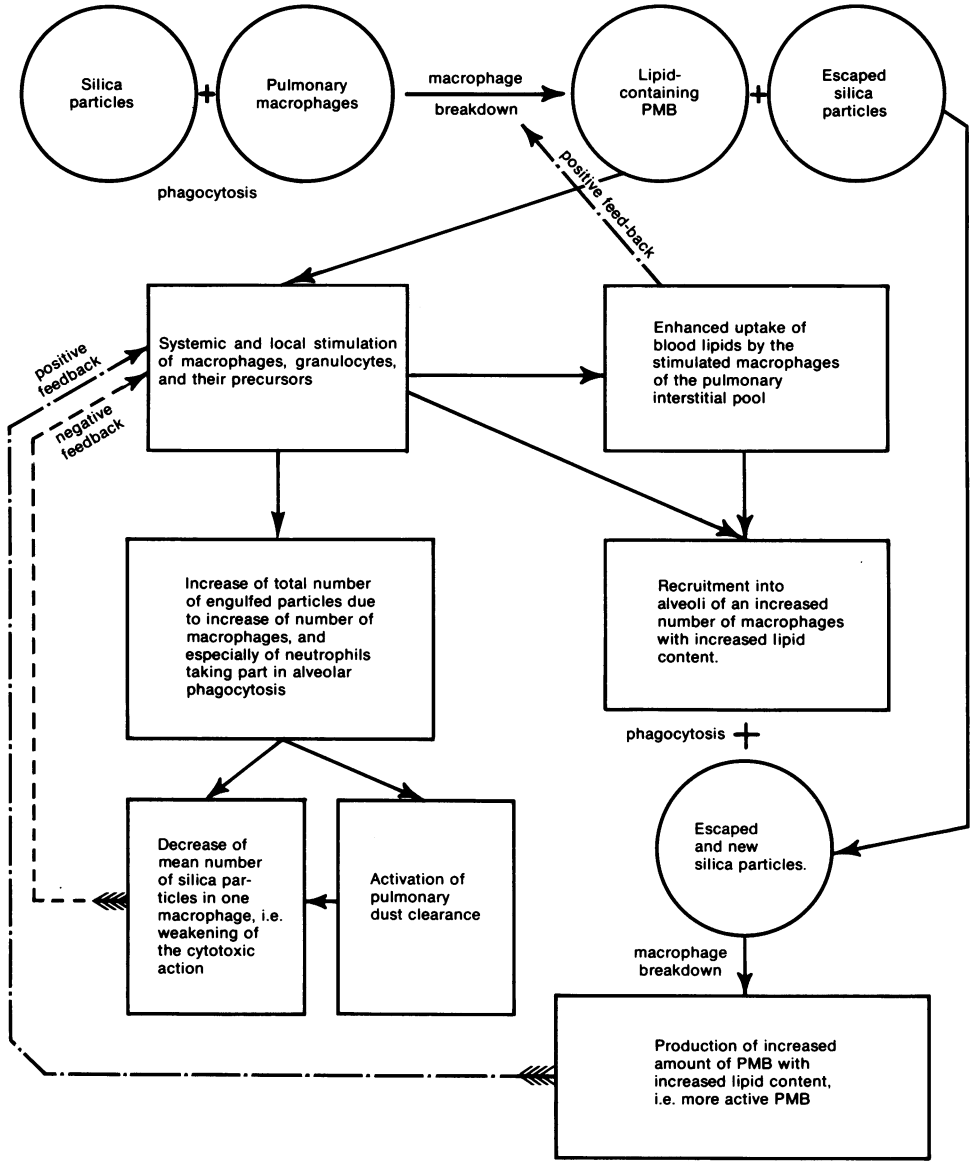

FIGURE 2. Control of the alveolar phagocytosis response to deposited silica particles by the macrophage breakdown products (PMB).

rine, or estrogens (*72*). This recruitment may be depressed under the influence of serotonine (*30*), a distant inflammation site (*73*), and glucocorticoids which also depress the phagocytizing ability of macrophages (*37, 74*). However, on the whole, the data accumulated hardly permit one to speak about any consistent system of ideas about the neurohumoral control of phagocytic responses in general and the alveolar phagocytic response in particular. We think that the autoregulation of this response can be considered to be the main one, while two points of conjugation of this autocontrol with the action of specialized control systems of the organism are possible. Some hormonal influences can probably change the AM resistance to damaging influences or else change the activity of engulfing of cytotoxic particles by AM; in both cases they influence the amount of PMB formed. Second, hormonal influences can change the reaction of different elements of the phagocyte response to the action of the same amount of PMB. In particular, we found that both the total number of cells recruited into the respiratory tract and the NL/AM ratio, as well as the influence of PMB on the number of dust particles phagocytized

by these cells, change considerably if the PMB is injected intratracheally 18 hr after an intramuscular injection of hydrocortisone.

Conclusions

An autocontrol mechanism depending on products of macrophage breakdown forms the basis of the operative adaptation of the pulmonary phagocytotic response to the number and properties of inhaled particles. The breakdown products stimulate the phagocytic cells and increase their output into the alveoli, simultaneously causing an AM/NL ratio shift which is favorable for the pulmonary clearance. They also help the recruitment of respective cell reserves, and signal the necessity of their replenishment by increased monocyto- and granulocytopoiesis. At least part of these effects is stimulated by the lipid components of PMB.

The autocontrol mechanism assures in the main the adequacy of the alveolar phagocytosis response. Although its continuous enhancement can, within certain limits, promote particle clearance, there always exists a danger of overstimulation, leading to an opposite result. Especially risky is the artificial stimulation of phagocytosis in response to quartz dust, after inhalation of which the high level of response is even without such stimulation provided by increased amounts of PMB. Besides, the enhancement of this response, connected with an additional use of cell reserves, is hardly favorable for the organism as a whole, especially when speaking about the ways of maintaining particle clearance efficient during many years and even decades. A search for physiological agents which increase the resistance of AM to damage and thereby help to increase the clearance efficiency along with a decrease of cell expenditure on it is indicated. When we deal with damage by silica particles to macrophages such increase of resistance means also a decrease of the factor stimulating silicotic fibrogenesis (*71, 75, 76*) and a favorable influence on other elements of the silicosis pathogenesis connected with a specific cytotoxicity of these particles. Experimental data showing that muscle training may provide a similar favorable influence lead to possible perspectives of this approach.

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